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Laparoscopic Ligation of the Infrarenal Vena Cava in Combination With Transfemoral Thrombin Infusion: A New Animal Model of Chronic Deep Venous Thrombosis

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Objective. *We describe a new technique to create an animal model of chronic venous thrombosis. The morphological and histological properties of the resulting thrombi are described.*
Methods. *Thirteen pigs underwent laparoscopic ligation of the infrarenal vena cava in combination with transfemoral thrombin infusion. After 1, 3, 6, 9, 12 and 15 days, respectively, two animals were killed and the thrombosed vein segments were explanted. After recording their weight and dimensions, the thrombi underwent histological examination by light microscopy.*
Results. *In all 13 cases, the procedure was completed laparoscopically and all 13 animals survived the procedure. While 12 pigs (92%) had an uneventful postoperative course, one animal died on the first postoperative night of an unknown cause. Autopsy revealed correct placing of the ligature with occlusive thrombosis of the inferior vena cava and the iliac veins in 12 animals, in one animal the ligature had been incorrectly placed around the origin of the right iliac vein with thrombosis limited to that vessel. Histological evaluation demonstrated mixed thrombi that showed increasing signs of organisation with advancing age.*
Conclusions. *Laparoscopic ligation of the infrarenal vena cava in combination with transfemoral thrombin infusion is a safe and reliable way to produce chronic venous thrombosis in an animal model. The resulting thrombi are comparable to human deep venous thrombosis in terms of extent, size and organisation process.*

Keywords: Deep vein thrombosis; Animal model; Laparoscopy.

Introduction

While there are *in vitro* models of acute thrombosis, the creation of a chronic, aging thrombus is dependent on *in vivo* conditions. This is the only way to reproduce the processes that change the thrombus with the passage of time. Several models of chronic as well as acute venous thrombosis have been described in a variety of animals. Generally, a combination of stasis with electrical,[1,2] mechanical[3] or pharmacological[4] stimulation of the vessel wall is used to create the thrombus. In older reports open ligation of the experimental vein is often performed to produce stasis leading to acute thrombosis. Depending on the part of the venous system that is investigated (i.e. vena cava or iliac veins), this necessitates a relatively big operation with the risk of major complications.[4] Recent reports describe the use of catheter-based techniques to induce venous stasis, reducing the operative trauma.[5–7]

We describe a new animal model of chronic deep venous thrombosis consisting of laparoscopic ligation of the infrarenal vena cava in combination with transfemoral thrombin infusion. The results of this technique as well as the histological properties of the resulting thrombi are reported.

Materials and Methods

All animal procedures and care were performed according to the German animal protection law and official permission to perform animal experiments was

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obtained. A total of 15 male domestic hybrid pigs aging 3–4 months were used in the study. Two pigs were used for preliminary experiments to evaluate the feasibility of laparascopic dissection and ligation of the inferior vena cava and 13 pigs were used for the actual study. The animals were kept in an animal care facility with ad libitum access to food and water for an accommodation period of 10 days. Twenty-four hours before the surgery, the animals were maintained on clear water and additionally bowel preparation was performed using a hyperosmolar solution (Endofalk, Dr Falk Pharma, Freiburg, Germany) dissolved in water.

On the day of the procedure, the animals were sedated with an intramuscular injection containing ketamine (30 mg/kg bw), atropine (0.05 mg/kg bw) and azaperon (2 mg/kg bw). They were then taken to the operating room where an intravenous catheter was placed and endotracheal intubation was performed. Anaesthesia was maintained by inhalation of enfluran 1.5%. All subjects breathed spontaneously and monitoring consisted of pulse oximetry. During the operation 500 ml of saline with the addition of 40 mg of pantozol and 5 mg/kg bw of gentamicin was slowly infused. The animals were placed on the operating table on their back in a supine position. After sterile prepping and draping a Verres-needle was inserted and a pneumoperitoneum was created by CO_2 insufflation up to a pressure of 12 mm mercury. The first trocar was positioned in the lower abdomen, then the laparoscope was inserted and two more trocars were placed in the left and right lower abdomen under visual control. A fourth trocar was only inserted in the left lower abdomen if it became necessary during the operation. If the bladder was full and protruded into the operating field, it was emptied by percutaneous puncture under laparoscopic control. The pig was then placed in a 30° Trendelenburg position by tilting the operating table. Using laparoscopic instruments, the small bowel was shifted in the upper abdomen and the sigmoid colon to the left side. The retroperitoneum was opened below the kidneys and the inferior vena cava identified. The vena cava was then carefully dissected out until it was possible to pass a laparoscopic forceps underneath it (Fig. 1). Using this forceps, a 1/0 silk suture was placed around the vein, then tightened and secured with three knots (Fig. 2), thereby occluding the inferior vena cava. The trocars were then removed and the incisions closed with interrupted sutures. In the two animals used for the preliminary experiments, the operation was terminated at this point.

In the 13 study animals, the medial circumflex femoral vein was dissected out in both groins and a 20 G catheter (Braun Melsungen AG, Melsungen, Germany) was inserted and forwarded 10 cm to reach the deep femoral vein. Intraluminal position of the catheter was confirmed by free aspiration of blood. Two hundred and fifty IU of bovine thrombin (Merck, Darmstadt, Germany) dissolved in 50 ml of saline were continuously infused at a rate of 6.25 IU/min over both catheters simultaneously. After completion of the infusion, the catheters were removed and the groin incisions closed with interrupted sutures.

Anaesthesia was then discontinued and the animals extubated. A patch releasing 50 μg/h of fentanyl was placed on the ear for postoperative pain control for 48–72 h. Additionally, the animals received 12.5 mg/kg bw of chlortetracycline for 3 days.

The two animals used for the preliminary experiments were sacrificed after 2 days. At autopsy the accuracy of application of the ligature was assessed and whether it occluded the inferior vena cava. The vena cava and iliac veins were examined for thrombus formation. In the study group, two animals were sacrificed and autopsy was performed after 1, 3, 6, 9, 12 and 15 days, respectively. The inferior vena cava below the ligature and both common iliac veins with the contained thrombus were removed en bloc (Fig. 3). The specimen was measured and weighed, then the common iliac veins were detached and measured and weighed separately. In order to minimise the effect of the ligature on the histological findings, the histological examination was performed on the mid section of the common iliac veins. A cross-sectional slice of each iliac vein and the contained thrombus at that site was cut and prepared for histological examination.

Specimens for histological examination were fixed in formaldehyde, then dried by application of alcohol solutions in increasing concentrations and finally embedded in paraffin. Using a microtome, slices with a thickness of 4 μm were obtained. These were mounted on cover glasses, stained using H&E, Elastica van Gieson and ferritin staining and then underwent histological examination by light microscopy.

Results

In all 15 animals, it was possible to complete the procedure laparoscopically and there was no instance of open conversion. In three cases percutaneous bladder puncture became necessary. In most animals control of the bowel and dissection and ligation of the inferior vena cava was readily achieved. Blood loss during the procedure was minimal. Table 1 gives an overview of the intraoperative data.

Autopsy in the two animals that underwent

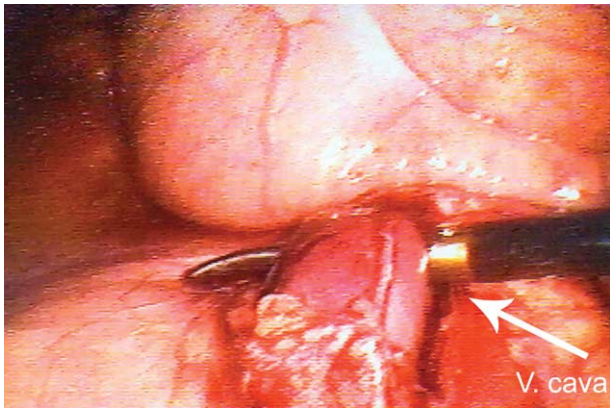

Fig. 1. Vena cava dissected and with laparoscopic forceps passed underneath it.

laparoscopic ligation without thrombin infusion revealed that the ligature was correctly placed and occluded the inferior vena cava below the renal veins in both animals. There was thrombus formation in the vena cava distal to the ligature, but no thrombus was found in the iliac veins on both sides.

All 13 study animals survived the operation. Twelve animals (92%) had an uneventful postoperative course with a normal level of activity and no signs of cardiac or respiratory dysfunction. One animal died during the first postoperative night. Autopsy including an evaluation of the pulmonary artery stems for evidence of pulmonary embolism revealed no gross pathological findings, so that the ultimate cause of death remained unclear. In 12 animals autopsy showed correct placing of the ligature with occlusion of the inferior vena cava and thrombus in the vena cava and the iliac veins. In one animal the ligature was incorrectly placed around the right common iliac vein just below its junction with the vena cava and thrombus was only present in the right iliac veins while the vena cava and the left iliac veins remained

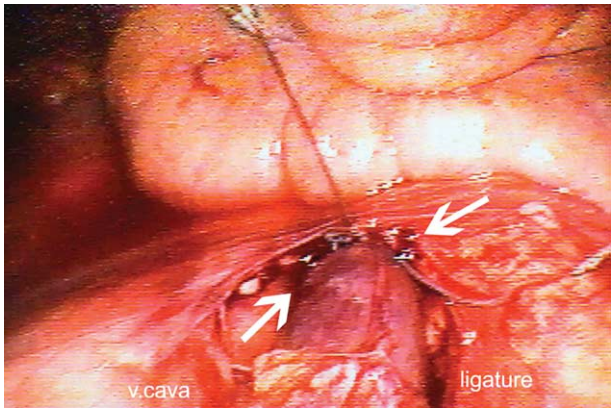

Fig. 2. Vena cava occluded by the ligature.

Fig. 3. Typical specimen of thrombotic vena cava and iliac bifurcation.

patent. Table 2 lists the dimensions and weights of the thrombotic vein specimens.

Histological examination by light microscopy revealed a mixed thrombus rich in red blood cells and platelets caught in a fibrin mesh at day 1 (Fig. 4). At the age of 3 days, the first signs of organisation appeared with proliferation of endothelial cells and beginning of scarce fibroblast and capillary infiltration of the thrombus. At days 6 and 9 the organisation of the thrombus proceeded with focal areas of fibroblast and capillary sprouting within the thrombus (Fig. 5), in addition collagen filaments became visible. The thrombi also contained areas of focal calcification at the edge, and additionally in the vessel wall an increasing number of granulocytes and macrophages were present. After 12 and 15 days, the thrombi showed signs of advanced organisation with extensive fibroblast, capillary and collagen infiltration of the thrombus with calcification and also focal recanalisation. The vessel wall was rich in granulocytes, lymphocytes and macrophages. Additionally, a

Table 1. Intraoperative data

Animal weight (kg)	Duration of operation (min)	Time until ligation of V. cava (min)	Blood loss (ml)
32 SE 8 (24–48)	70 SE 34 (35–135)	40 SE 15 (23–72)	32 SE 33 (0–100)

The numbers represent the mean value and standard error (SE), the numbers in parenthesis are the minimum and maximum values.

proliferation of the smooth muscle cells of the media as well as a fibrous thickening of the intima was present (Fig. 6).

Discussion

Most animal models of venous thrombosis described in the literature rely on a combination of venous stasis with electrical,[1,2] mechanical[3] or pharmacological[4] damage to the venous endothelium to induce thrombosis. While the main goal of producing a thrombus can be achieved by those means, there are several limitations. In many of the studies, focal thrombosis of a single vessel is produced and, depending on the animal used, the thrombosed vessel is relatively small.[3,8–11] This is somewhat different from deep venous thrombosis in humans, where larger masses of thrombus extend in continuity within the axial veins of the legs. Furthermore, the small calibre of the veins precludes the study of certain interventional treatment modalities because of access problems. Larger models are often quite difficult to create,[12] and in addition the procedure to produce the venous stasis can carry a significant mortality.[4]

Another point of criticism is the sometimes extensive endothelial damage used in some studies as part of the thrombosis induction. Investigations on the aetiology of venous thrombosis[13,14] suggest that major endothelial damage usually is not involved in the development of DVT in a clinical setting. Thrombi associated with such endothelial damage might differ from their clinical counterparts in terms of their structure, form of organisation and adherence to the vessel wall, thereby making the evaluation of thrombolytic therapies difficult.

Since the complex mechanisms causing venous thrombosis in humans are not fully understood, it is difficult to create a model of venous thrombosis that truly resembles the situation in human DVT. As reported by different authors[1,4] and also confirmed in our preliminary experiments, venous stasis alone does not reliably lead to continuous, occlusive venous thrombosis. Several authors believe that the generation of thrombin in the presence of vascular stasis is an initiating event during thrombus formation.[13–15] It seems plausible to mimic this situation in an animal model of venous thrombosis and a number of groups have successfully utilized this concept in their work.[6,7,16] Although any animal model of venous thrombosis creates an artificial situation, the injection of thrombin, which occurs naturally during thrombus formation, might be more realistic than the application of extensive endothelial damage or the insertion of a foreign body into the vein.

Additionally, many of the above mentioned technical problems have been addressed in the recent literature. One major improvement was the use of interventional, catheter-based techniques to create venous stasis.[5–7,17] This has eliminated the need for open ligation of the iliac veins or the vena cava, which especially in pigs, who react poorly to stress, can carry

Table 2. Weight and length of the thrombotic vein specimens

Animal	Thrombus age (days)	Weight IVC (g)	Length IVC (mm)	Weight LCIV (g)	Length LCIV (mm)	Weight RCIV (g)	Length RCIV (mm)
1	1	4	35	4	46	3	33
2	1	5	36	0.5	32	1	34
3	3	4	36	3	36	2	25
4	3	6	41	2	32	1	29
5	6					0.5	30
6	6	6	43	2	37	3	31
7	9	2	20	2	21	1	24
8	9	3	34	2	24	2	30
9	12	2	21	0.5	21	3	35
10	12	4	40	0.5	23	0.5	23
11	15	2	15	3	32	2	32
12	15	2	21	0.5	21	1	29
Mean, SD		3.6 SD 1.6	31.1 SD 9.9	1.8 SD 1.2	29.5 SD 8.2	1.6 SD 1	29.6 SD 3.9

In animal 5, the ligature was falsely placed around the origin of the right common iliac vein instead of the inferior vena cava, so that in that animal only the right common iliac vein was thrombosed. IVC, inferior vena cava; LCIV, left common iliac vein; RCIV, right common iliac vein, SD, standard deviation.

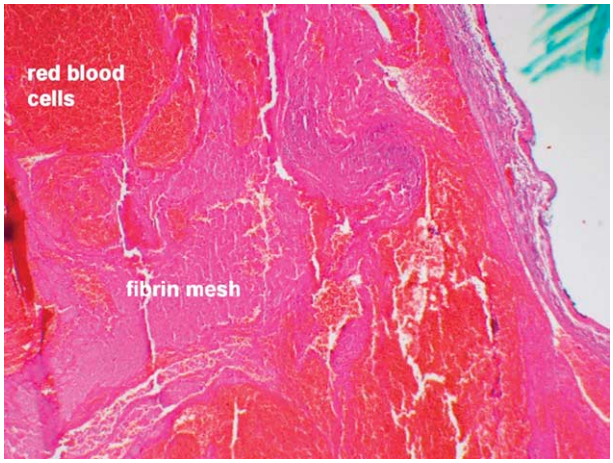

Fig. 4. Three day old thrombus with red blood cells caught in a fibrin mesh (Elastica van Gieson staining, original magnification 40×).

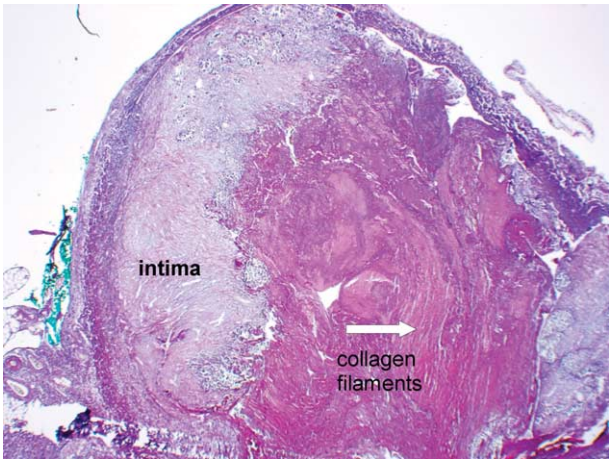

Fig. 6. Twelve day old thrombus demonstrating advanced signs of organisation with medial hyperplasia, fibrous thickening of the intima and abundant collagen filaments (H&E staining, original magnification 12, 5×).

a mortality as high as 33%.[4] However, the use of these catheter-based techniques requires the availability of a C-arm in the operating room, a facility that might not be available in may animal laboratories. Interventional skills are necessary and a new set of sheaths, guide-wires and catheters is needed for every procedure, adding to the costs of the model.

Another problem is the creation of a chronic thrombosis model. While it is feasible to produce venous stasis by balloon catheter occlusion for a short period of time in models of acute venous thrombosis,[6] the prolonged stasis needed in a chronic model is more difficult to achieve with balloon migration or deflation and infection of the catheter being potential problems. Tacke *et al*.[7,17] used the combination of coil occlusion of the common iliac vein with thrombin infusion in their animal model. Although they successfully produced occlusive venous thrombi of the common iliac vein in all their study animals, they also describe coil migration and pulmonary embolism as potential problems. Lin *et al*.[5] recently described a porcine model of chronic deep venous thrombosis where they deployed a tapered graft attached within a self-expanding nitinol stent to the iliac veins, thereby creating venous stasis and subsequently thrombus formation. While thrombus formation occurred in all animals in this model, complete thrombotic occlusion of the iliac vein was never found. Additionally, a caval filter had to be placed in each animal to prevent pulmonary embolism.

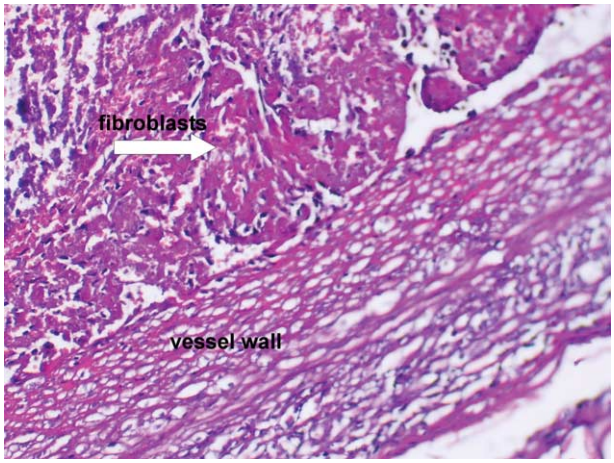

Fig. 5. Six day old thrombus showing fibroblast sprouting from the vessel wall (H&E staining, original magnification 200×).

The laparoscopic technique used in our model provides a minimally invasive way to create venous stasis, and in addition has the advantage of being a straight-forward and inexpensive procedure. All animals survived the operation, and only one animal died in the postoperative course of an ultimately unknown cause. All animals, including the one, which unexpectedly died during the first postoperative night, recovered quickly from the procedure and did not show prolonged signs of distress or pain. This underlines the minimal invasive character of the laparoscopic operation. The instruments can be reused after sterilisation, thereby keeping the costs of the model low. The placement of a ligature around the vena cava ensures venous stasis without causing extensive damage to the venous endothelium and without the need to place a foreign body within the vein. Furthermore, by leaving the lumbar venous branches patent, complete flow arrest was avoided; a factor which is believed to be important in the development of histologically realistic venous

thrombi.[16] The combination of the venous stasis with transfemoral infusion of thrombin resulted reliably in occlusive thrombus formation in both iliac veins and the vena cava. Clinically significant pulmonary embolism was not observed in any of the animals despite this extensive thrombus formation. The dosage and infusion rate of thrombin used in our model were based on a study by Roy *et al*.[6] who used balloon-catheter occlusion and thrombin infusion to create an animal model of acute venous thrombosis. They were able to produce venous thrombosis extending from the femoral to the iliac veins in all their study animals.

In our model, the vena cava was occluded completely and the thrombosed vein segments explanted for further examination. For the evaluation of thrombolytic therapies or the examination of thrombosis induction it might be desirable to just narrow the vein or to not compromise flow altogether and just afflict a defined damage to the vein wall. Once the laparoscopic dissection of the vena cava is accomplished, it would be simple and technically unproblematic to place a band around the vessel instead of the suture and to create a defined stenosis by tightening of the band. It is also imaginable to laparoscopically apply some sort of stimulus to the vein wall in order to induce thrombosis. In both cases the laparoscopic technique would have the advantage of eliminating the need to insert a foreign body into the vein.

Histological examination of the thrombi produced in our model showed on day 1 a mixed thrombus rich in red blood cells and platelets caught in a fibrin mesh. The 3 day old thrombi showed first signs of organization and this process continued over time with the 15 day old thrombi showing advanced organization characterized by the presence of collagen filaments, fibroblasts, capillaries and focal recanalisation as well as focal calcification. This is in agreement with other studies that demonstrated histologically realistic thrombi after venous flow impairment and thrombin infusion.[7,16,17] The finding of areas of focal calcification in the 12 and 15 day old thrombi is somewhat surprising. Whereas the calcification of venous thrombi is also described in humans,[18–20] one would expect it in older thrombi. There is, however, no concrete information in the literature on when the first, focal signs of calcification are seen in human venous thrombi. This makes it difficult to interpret the findings in the animal model, but it might be that this specific process of thrombus organisation takes place faster in pigs than in humans. The other changes in the structure and the fibrillar architecture of the experimental thrombi observed over time resembled the ones described in human venous thrombosis.[21]

In summary, laparoscopic ligation of the infrarenal vena cava in combination with transfemoral thrombin infusion is a safe, cheap and reliable way to produce an animal model of chronic deep venous thrombosis. It should be also feasible in other animals than pigs, provided they are large enough to allow adequate laparoscopic visualization and dissection. The procedure has a low complication rate and is tolerated well by the animals. The size and extent of the produced thrombi as well as their structure and aging process seem to be comparable to the findings in human venous thrombosis.

Acknowledgements

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